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(54) Title: SMILAGENIN AND ANZUROGENIN-D AND THEIR USE		
(57) Abstract The invention discloses the use of a smilagenin and anzurogenin-D in the treatment of cognitive disfunction and similar conditions. Methods of treatment, and pharmaceutical compositions are also disclosed.		

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SMILAGENIN AND ANZUROGENIN-D AND THEIR USE

The present invention relates to smilagenin and anzurogenin-D and their use in treating cognitive disfunction and allied conditions; and to compositions for use in such treatments. The invention is also concerned with the treatment of conditions that are characterised by a deficiency in the number or function of membrane-bound receptors. In the following, the present invention will be described principally with reference to the treatment of Alzheimer's disease (AD) and senile dementia of the Alzheimer's type (SDAT), where deficiencies in a number of receptor types have been demonstrated. However, it is to be understood that the present invention relates generally to the treatment of conditions attributable to intrinsic pathological conditions and/or exposure to adverse environmental conditions these conditions being characterised by a deficiency in the number or function of membrane-bound receptors or a deficiency in transmission at the junctions between neurones or at the junctions of neurones and effector cells.

Conditions of the type mentioned above include Parkinson's disease, Lewi body dementia, postural hypotension, autism, chronic fatigue syndrome, Myasthenia Gravis, Lambert Eaton disease, diseases and problems associated with Gulf War Syndrome, occupational exposure to organophosphorus compounds and problems associated with ageing.

Alzheimer's disease (AD) and senile dementia of the Alzheimer's type (SDAT) are grave and growing problems in all societies where, because of an increase in life expectancy and control of adventitious disease, the demographic profile is increasingly extending towards a more aged population. Agents which can treat, or help in the management of, AD/SDAT are urgently required.

Age-associated memory impairment (AAMI) is a characteristic of older patients who, while being psychologically and physically normal, complain of

memory loss. It is a poorly defined syndrome, but agents which are effective in treatment of AD/SDAT may also be of value in these patients.

5 Research into AD/SDAT is being carried out by traditional and
conventional medical research methods and disciplines. In conventional
medicine, there are several approaches to the treatment of AD/SDAT. It is
known that the biochemical processes subserving memory in the cerebral cortex
are (at least in part) cholinergically-mediated. Those skilled in the art will
10 know that "cholinergically mediated" mechanisms may be directly attributable to
acetylcholine acting on receptors, and these are direct effects. Other, clinically
useful effects may also be caused by modulation of release of acetylcholine
from pre-synaptic nerve endings or inhibition of enzymes that destroy
acetylcholine. These modulating factors may be exerted through neurones
where the mediator is non-cholinergic; these are referred to as indirect effects.
15 Some attempts at treatment have focussed on the role of other mediators such as
5-hydroxytryptamine, which is a mediator in other areas of brain, such as the
mid-brain nuclei. However, since fibres from these areas are projected forward
into the cerebral cortex where the primary transmitter is acetylcholine, attention
has focussed on the management of this mediator in the search for appropriate
20 therapeutic agents.

Cholinergic strategies for the treatment of AD/SDAT have been directed
at several points along the pathway of formation, synaptic release and removal
of released acetylcholine.

25 One approach involves treatment with high doses of lecithin and other
precursors of acetylcholine. This is of limited use in producing sustained
improvements in cognitive performance.

30 Another approach involves the use of vegetable drugs such as Polygalae
root extract, which has been shown to enhance choline-acetylcholine transferase

(CAT) activity and nerve growth factor (NGF) secretion in brain. Oral administration of NGF has no effect on central nervous system neurons because it is a high molecular weight protein that cannot pass through the blood-brain barrier. However, agents which can pass through the blood-brain barrier and have a stimulating effect on NGF synthesis in the central nervous system have been proposed for the improvement of memory-related behaviour.

The results of a third clinical approach, which uses cholinesterase inhibitors such as tacrine hydrochloride, have been marginally more positive than the above. Substances obtained from plants used in Chinese and Western medicine, for example huperzine, galanthamine, and physostigmine have all been shown to be of some – although limited – benefit in the treatment of AD/SDAT in clinical studies and also in laboratory models. All of these substances are inhibitors of acetylcholine esterase (AChE). In patients with AD/SDAT, there may be reduced synthesis of acetylcholine (ACh), reduced efficiency in release of ACh from presynaptic stores, and a decrease in the number or function of postsynaptic (M_1) receptors. Reductions in pre-synaptic M_2 receptors have also been shown. The beneficial effect of AChE inhibitors is attributed to enhancement of acetylcholine levels at synapses in brain by slowing down the destruction of released transmitter.

Compositions which modulate cholinergic function are known to affect memory and recall. For example, nicotine stimulates nicotinic acetylcholine receptors, and the short lived memory enhancing effects of cigarette smoking are thought to be due to the effect of nicotine. Scopolamine, an antagonist of acetylcholine, will produce amnesia and impaired cognitive function manifesting in psychomotor tests as a prolongation of simple reaction times, possibly as a result of impaired attention, and is used for this purpose as an adjunctive analgesic treatment. The amnesic effect of scopolamine can be antagonised by nicotine.

There are two families of nicotinic receptor subtypes (α and β), and each includes four subgroups which differ in ligand specificity. The role of nicotinic receptors in the CNS is not well understood at the molecular level. It is possible that agents binding to nicotinic receptors may modify the rate of turnover at muscarinic receptor sites in brain. Nicotinic receptors are ligand-gated ion channels, and their activation causes a rapid (millisecond) increase in cellular permeability to Na^+ and Ca^{++} , depolarisation and excitation.

Another class of cholinergic receptors can be stimulated by muscarine. Such muscarinic (M) receptors are G protein-coupled receptors. Responses of muscarinic receptors are slower; they may be excitatory or inhibitory. They are not necessarily linked to changes in ion permeability. Five types of muscarinic receptors have been detected by cholinergic receptor cloning, and are designated as m_1 - m_5 . Pharmacological effects are associated with four of the cloned receptors and they are designated as M_1 - M_4 based on pharmacological specificity.

Using specific receptor proteins and monoclonal antibodies, it has been possible to further localise muscarinic receptors in brain as m_1 (postsynaptic) and m_2 (presynaptic). In heart, M_2 receptors are postsynaptic. Presynaptic muscarinic receptors are thought to be inhibitory, the binding of ACh to these receptors attenuating the release of further ACh to provide a negative feedback mechanism for ACh release. Selective M_2 receptor antagonists which are preferentially distributed to the brain may therefore be useful in treating Alzheimer's disease.

It is known that, in disease states such as AD/SDAT, there is general neuronal loss and deficits in cholinergic nerve function. It has been speculated that the high affinity nicotinic binding sites in the remaining cholinergic neurons might be converted to low affinity binding sites in treating such diseases, thereby sustaining transmitter release. By lowering the affinity of the nicotinic

binding sites, a quick desensitising process is avoided.

Agonist activation at nicotinic receptors in brain has rapid onset and offset. A decreased affinity of the nicotinic receptors will reduce the desensitisation process. Schwarz R.D. et al (J. Neuro Chem 42, (1984), 1495-8) have shown that nicotine binding sites are presynaptically located on cholinergic (and also 5-hydroxytryptaminergic and catecholaminergic) axon terminals. A change in high affinity binding sites on AD/SDAT may also induce a change in the modulatory effect the nicotinic binding sites may have on other transmitter systems.

Presynaptic cholinergic mechanisms are also under inhibitory control by GABAergic neurons and this inhibition is thought to be intensified in AD/SDAT. Removal or reduction of this inhibition intensifies presynaptic cortical cholinergic activity and enhances cognitive processing.

The interactions of interneuronal fibres innervated by nicotine (reducing binding affinity), and dis-inhibition of GABAergic fibres both have a presynaptic locus.

This is a simplistic model of central transmission, but provides a framework for understanding the attempts which have been made to increase the effective concentration of acetylcholine in central synapses. This further illustrates the concept of direct and indirect action. There are disadvantages attaching to the three conventional therapeutic approaches to AD/SDAT treatment mentioned above: ACh precursor supplementation, agonist replacement and acetylcholine esterase inhibition. These treatments may result in a short-term increase in the availability of ACh which may activate feedback mechanisms resulting in the desensitisation of postsynaptic receptors. On theoretical grounds, long term benefits would not be predicted and when treatment is interrupted, any benefits in management of AD/SDAT and AAMI

disappear and the condition may even be aggravated.

5 It has been shown that a compound with M_1 agonist and M_2/M_3 antagonist activity improved cognitive performance in SDAT patients (Sramak et al, Life Sciences vol. 2, No. 3, 195-202, 1997). However, this compound causes unacceptable cholinergic side effects, such as fatigue, diarrhoea and nausea.

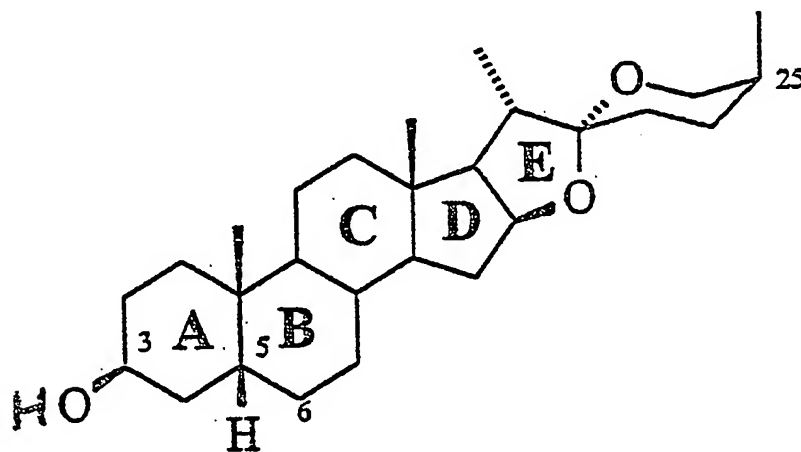
10 A more radical approach to AD/SDAT and AAMI aims to increase the number of postsynaptic (M_1) receptors, in brain. It is known from Chinese Patent No. CN1096031A, that sarsasapogenin (SaG) can up-regulate M_1 cholinergic receptors and also down-regulate (i.e. move towards normal levels of) β -adrenergic receptors, the number of which may be pathologically-raised in AD/SDAT.

15 Patent applications have been published which claim the usefulness of a number of steroid sapogenins having spirostane, furo-spirostane, spirosolane or solanidine structures in the treatment of diseases including SDAT. Two patent publications are of particular relevance here: Chinese patent publication No
20 CN1096031A claims the use of the spirostane sapogenin, sarsasapogenin, in the treatment of SDAT. The disclosure in this document, however, is brief. The other document of relevance is patent publication DE 4303214A1 which claims the use of a very wide range of saponins and sapogenins in the treatment of a whole range of diseases that the inventors consider to be of viral origin. This
25 disclosure is however of dubious value in that it is well recognised that there is no infective element to a very large number of the conditions that are characterised by deficient synaptic transmission and thus the basic premise of the alleged invention is flawed. In addition they present no data of any kind that allows one skilled in the art to be able select a preferred compound from the
30 large number that are claimed.

The inventors have found that smilagenin (SMI) and anzurogenin D (AZD) exhibit the ability to regulate receptors. In particular, these compounds – and especially SMI – have been found to increase the number of M2 receptors in the brain. Thus, according to one aspect of the invention, there is provided the use of smilagenin and/or anzurogenin D in the manufacture of a medicament for the treatment of a condition characterised by a deficiency in postsynaptic membrane-bound receptor number or function.

Those skilled in the art will be aware of the relationship between saponins and their sapogenins, and that the desired effects of sapogenins can be exhibited in patients by administration of the corresponding saponins, or a mixture thereof. Hydrolysis of at least a proportion of saponin occurs in the gastrointestinal tract. The skilled man will also be aware of the epimerisation of certain sapogenins under conditions of acid hydrolysis.

The sapogenins of interest in this invention are of the following general formula:



With reference to this general formula, SMI and AZD have the structure indicated in the Table below:

Compound	A/B ring Cis/Trans/ unsaturation	C25 methyl stereochemistry (R or S)	Hydroxyl group(s) on Spirostane ring
Smilagenin	Cis	R	3 β -OH
Anzurogenin-D	Trans	R	3 β -OH, 5 α -OH, 6 β -OH

5

The saponins and sapogenins of interest in the present invention occur naturally in a range of plant species, notably from the genera Smilax, Asparagus, Anemarrhena, Yucca and Agave. The species presently of greatest interest include Smilax regelii Kilip & Morton - commonly known as Honduran sarsaparilla; Smilax aristolochiaefolia Miller - commonly known as Mexican sarsaparilla; Smilax ornata Hooker - commonly known as Jamaican sarsaparilla; Smilax aspera - commonly known as Spanish sarsaparilla; Smilax glabra Roxburgh; Smilax febrifuga - Kunth - commonly known as Ecuadorian or Peruvian sarsaparilla; Anemarrhena asphodeloides Bunge; Yucca schidigera Roez l ex Ortgies; and Yucca brevifolia Engelm.

15

According to a further aspect of the present invention, there is provided a pharmaceutical composition having cognitive function enhancing properties which comprises an effective amount of smilagenin and/or anzurogenin D.

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In another aspect, the invention provides a pharmaceutical composition having cognitive function enhancing properties which comprises an effective amount of smilagenin and/or anzurogenin D in the form of an extract derived from a plant of the genus Smilax, Asparagus, Anemarrhena, Yucca or Agave.

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It will be appreciated that the invention embraces within its scope the use

of the compositions defined above. Thus, according to a fifth aspect, the present invention provides a method of enhancing cognitive function which comprises administering to a human or animal an effective dosage of a composition of the invention.

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The invention also provides a method of enhancing cognitive function in a human or non-human animal, which comprises administering an effective dose of smilagenin and/or anzurogenin D.

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As used herein, the term "cognitive function" refers to functions such as thinking, reasoning, remembering, imagining and learning.

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In identifying compounds that would have use in the treatment of SDAT and other diseases characterised by reductions in receptor numbers or synaptic transmission, the inventors have given consideration to the need to identify compounds that would have the desired effect but would be devoid of any oestrogenic effects, as these would be unacceptable, particularly in male patients. A number of the compounds claimed to have activity in patent application DE 4303214A1 have marked oestrogenic activity and are therefore unacceptable. Smilagenin and anzurigenin D, however, do not display oestrogenic activity. In addition these two compounds were tested at other steroid receptors and neither compound was found to have any activity at any of the following receptors:

20

25

Progesterone
Glucocorticoid
Testosterone

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The selected compounds have also been tested for their activity in a number of in-vitro assays. The assays/experiments that were considered of key importance in determining possible activity in the elevation of membrane bound

receptor numbers were as follows:

1. Chinese hamster ovary (CHO) cells transfected with the a DNA fragment coding for a muscarinic receptor. The cell line used for the majority of the experiments was a cell line expressing the m2 receptor.
2. The effects of muscarinic receptor expression in cultured cell lines of neuronal origin were investigated.
3. Cultured cardiac muscle cells obtained from neonatal Sprague Dawley rats. The cardiac muscle cells express muscarinic receptors, typically m2. The level of these receptors falls on prolonged culture and the effects of compounds of interest in preventing the fall in receptor numbers was investigated.

The methods and the results of these experiments are now described in turn.

1 CHO cell line experiments

The effects of various compounds on the expression of m2 receptors on CHO cells transfected with DNA for the m2 receptor were investigated. Receptor numbers were assayed using tritiated QNB binding and subtracting non-specific binding. Compounds were dissolved in DMSO and DMSO was used as a control. Compounds were tested at a range of final concentrations. Compounds were also tested in the presence and absence of tamoxifen to try to distinguish an oestrogen receptor mediated mechanism. The results are summarised in the Table 2 below, where the compounds used in the invention appear in bold, and data on other sapogenins is given for comparative purposes:

Table 2 Effects of compounds on the expression of m_2 receptors on CHO cells

Compound	Molar concentration of compound	Effect on receptor expression – given as % increase compared to control (negative values in brackets)
Sarsasapogenin	10^{-5}	34
	10^{-6}	(14)
Anzurogenin D	10^{-5}	22
	10^{-6}	(26)
Sisalgenin	10^{-5}	NS
	10^{-6}	NS
Smilagenin	10^{-5}	57
	10^{-6}	18
Diosgenin	10^{-5}	NS
	10^{-6}	NS
Ruscogenin	10^{-5}	(22)
	10^{-6}	NS
Tigogenin	10^{-5}	NS
	10^{-6}	NS

NS = No significant effect

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Thus the experiments indicate that smilagenin and anzurogenin D were able to increase the number of muscarinic receptors expressed on the surface of CHO cells cultured in-vitro. The effect was not antagonised by tamoxifen, indicating that the mechanism involved did not involve the oestrogen receptor.

5

2 Effects of compounds on cell survival

Other in vitro assays have been employed to establish the effects of smilagenin and anzurogenin D. In particular various neuroblastoma cell lines including SKN-SN and SH-SY5Y cells as well as phaeochromocytoma cell lines have been cultured in vitro in the presence of β -amyloid fragments or serum depletion. A number of techniques to demonstrate the effectiveness of the compounds in protecting the cultured cells were investigated. These techniques included Trypan blue exclusion, chemiluminescence and release of lactate dehydrogenase. Of most interest was the observation that incubation of cells, in particular PC12 cells, with β -amyloid reduced the number of muscarinic receptors measured using radio-labelled ligand binding techniques. This reduction in receptor numbers was found to be ameliorated by smilagenin and by anzurogenin D.

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3 Effects of compounds on cultured cardiac muscle cells.

Cardiac muscle cells were isolated from the ventricular muscle of neonatal Sprague Dawley rats using standard techniques. Cells were cultured in vitro and muscarinic receptor numbers expressed on cell surfaces membrane fragments after homogenisation of cells harvested at various time points were estimated using specific binding of tritiated QNB. Preliminary experiments demonstrated that the number of receptors expressed tended to decline after 10 days of culture. The experiments were therefore designed to investigate the effects of the various compounds in inhibiting this decline in receptor numbers.

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The results of these experiments are summarised in Table 3, where the compounds used in the invention appear in bold, and data on other sapogenins is given for comparative purposes:

5 **Table 3: Effects of various compounds on muscarinic receptor expression on cultured cardiac muscle cells**

	Compound	Concentration of compound causing a significant increase in number of receptors expressed on neonatal cardiac muscle after 10 days in vitro culture
	Diosgenin	NS
10	Anzurogenin D	10^{-6}M
	Ruscogenin	NS
	Sarsasapogenin	10^{-5}M
	Tigogenin	NS
	Astragaloside	10^{-5}M
15	Smilagenin	10^{-6}M

NS = No significant effect

20 It is speculated here that the effect of the active compounds claimed in this patent may operate through an effect on G protein and that the effects on receptor numbers are secondary to an effect on G-protein. When a membrane bound G-protein linked receptor is stimulated two basic sets of events are initiated: the effector response; and the internalisation of the receptor. The

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subsequent processing of the receptor to the state where it is again in a form on the cell surface or other membrane surface where it can interact with another receptor ligand appears to be subject to a number of factors. A number of these factors or mechanisms appear to be G-protein linked. There is evidence that activation of m_3 receptors may have an effect on G-protein expression or levels. It is speculated that the actions of the compounds described in this patent may due to an interaction in the processes of receptor regeneration, G-protein linkage or G-protein homeostasis.

10 An alternative hypothesis is that the compounds are increasing the synthesis or release or a decreased rate of degradation of neurotropic factors such as brain derived growth factor and/or nerve growth factor. These effects on growth factors might be due to an effect of the compound on a cytosolic or nuclear receptor or the binding of a compound to a promoter region with a consequent effect directly on the rate of production of mRNA for the growth factor or as a consequence of increasing the production of another material factor such as G-protein or finally the effects may be secondary to an effect on receptor or G-protein procession.

20

CLAIMS:

1. The use of smilagenin in the manufacture of a medicament for the treatment of a condition characterised by a deficiency in postsynaptic membrane-bound receptor number or function.
2. The use of anzurogenin D in the manufacture of a medicament for the treatment of a condition characterised by a deficiency in postsynaptic membrane-bound receptor number or function.
3. A pharmaceutical composition having cognitive function enhancing properties which comprises a pharmacologically effective amount of smilagenin and/or anzurogenin D.
4. A pharmaceutical composition having cognitive function enhancing properties which comprises a pharmacologically effective amount of smilagenin and/or anzurogenin D in the form of an extract derived from a plant of the genus Smilax, Asparagus, Anemarrhena, Yucca or Agave.
5. A method of enhancing cognitive function which comprises administering to a human or animal an effective dosage of a composition as claimed in claim 3 or 4.
6. A method of enhancing cognitive function in a human or non-human animal, which comprises administering an effective dose of smilagenin and/or anzurogenin D.
7. A method of enhancing cognitive function in a patient suffering from age-related cognitive disfunction, which comprises administering to the patient a pharmacologically effective dose of smilagenin and/or anzurogenin D.

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8. A method as claimed in claim 5 or 6, which is for the treatment of Alzheimer's disease or a senile dementia of the Alzheimer's type.

5 9. A method as claimed in claim 5, 6, 7 or 8, substantially as
hereinbefore described.

10. A composition for use in a method as claimed in any one of claims 5 to 9, substantially as hereinbefore described.

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<p>(54) Title: SMILAGENIN AND ANZUROGENIN-D FOR THE TREATMENT OF ALZHEIMER'S DISEASE (57) Abstract The invention discloses the use of a smilagenin and anzurogenin-D in the treatment of cognitive dysfunction and similar conditions. Methods of treatment, and pharmaceutical compositions are also disclosed.</p>		

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CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakhstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 99/00960

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K31/58 A61K31/70

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	N. YI ET AL.: "Sarsasapogenin: Mechanism in treating senile dementia" PROCEEDINGS OF THE SIXTH INTERNATIONAL SYMPOSIUM OF THE INTERNATIONAL ISOTOPE SOCIETY, PHILADELPHIA, 1997, pages 315-320, XP002117140 the whole document	1-10
X	STN INTERNATIONAL. FILE CHEMICAL ABSTRACTS. AN=123:266081, XP002117141 abstract & CN 1 096 031 A (SHANGHAI NO. 2 MEDICAL UNIVERSITY) cited in the application --- -/--	1-10

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "B" document member of the same patent family

Date of the actual completion of the international search

30 September 1999

Date of mailing of the international search report

13/10/1999

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
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Authorized officer

Orviz Diaz, P

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 99/00960

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DE 43 03 214 A (MARKS WOLFGANG) 11 August 1994 (1994-08-11) cited in the application page 6, line 15-26; claims 1,14 ----	1-10
E	WO 99 16786 A (WANG BINGJI ;DONG JUNXING (CN); MA BEIPING (CN); INST OF RADIATION) 8 April 1999 (1999-04-08) abstract -----	1-10
A	G. BLUDEN ET AL.: "Steroidal sapogenins from leaves of Agaveae species" STN INTERNATIONAL. FILE BIOSIS. AN=1979:172390, XP002117142 abstract -----	1-10

INTERNATIONAL SEARCH REPORT

International application No.

PCT/GB 99/00960

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 5-10
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 5-10
are directed to a method of treatment of the human/animal
body, the search has been carried out and based on the alleged
effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such
an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all
searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment
of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report
covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is
restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 99/00960

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
DE 4303214 A	11-08-1994	NONE	
WO 9916786 A	08-04-1999	CN 1212966 A	07-04-1999
		AU 9336098 A	23-04-1999